221P ENHANCED PROLIFERATION AND MIGRATION OF PRIMARY HUMAN DERMAL FIBROBLASTS INDUCED BY VARIOUS GROWTH FACTORS: IMPLICATIONS FOR DERMAL REPAIR

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During wound repair, a key process is one of cellular migration both into and over the wound bed. This is a complex process subject to precise control by a number of guidance cues (Britland *et al*, 1996). One such cue is the presence of chemotaxic stimuli such as growth factors. Growth factors are polypeptide molecules who through interaction with specific receptors on the cell surface regulate the recruitment of cells whose functions are believed necessary to ensure a satisfactory wound response (Moulin, 1995; Steed, 1997). This study examines the effect of acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and hepatocyte growth factor (HGF) on the proliferation and migration of primary human dermal fibroblasts (HDF).

HDF from passage 1-5 were plated out at a density of 1 x 10^4 cells/cm^2 using Hams F10 nutrient medium containing 1.2g/L $NaHCO_3$, 5% foetal bovine serum, 100umits/ml penicillin and 100µg/ml streptomycin. After 24 hours cell counts were performed and media was replaced with growth media containing aFGF (10ng/ml), EGF (10ng/ml), PDGF (5ng/ml) and HGF (5ng.ml). Control was growth medium without presence of additional growth factors other than those present in the serum supplement. Cell counts were made on days 1, 3, 6, 9, 11, 14 and expressed as a percentage of the initial cell count. For analysis of cell motility, cells were viewed using a CCD digital camera attached to a phase contrast microscope. Cultures were filmed for 24-72 hours at a rate of 8 frames per hour, during which time temperature of the medium was maintained at 37°C by a temperature control unit. Time-lapse films were converted into a series of still images and movement of cells was plotted using a macro developed for Scion Image. Co-ordinates of cell movement were then entered into a macro in Microsoft Excel which calculated arithmetically various aspects of cell behaviour such as velocity, persistence and total distance travelled.

Proliferation of HDF was found to be accelerated by aFGF, EGF and PDGF, this effect was marked with aFGF which induced more than a 10-fold increase [1200.8 ± 9.7 (mean ± s.e.mean); n=6) in cell numbers over 14 days, compared to only a 5 -fold increase (530.3 ± 9.4; n=6) observed in control cultures. Rate of cell migration was accelerated by aFGF, EGF and PDGF with mean velocities (µm/hour) of 4.6 ± 0.3 (n=15), 4.4 ± 0.4 (n=14) and 4.1 ± 0.6 (n=12) respectively compared to velocity in control cultures of 2.9 ± 0.2 (n=10). HGF did not demonstrate significant effects on either proliferation or growth of cells.

The results in the present study demonstrate the ability of aFGF, EGF and PDGF to enhance the proliferation and migration of HDF. *In vitro* studies investigating the role of growth factors on cell behaviour will provide important information on how proliferation and migratory behaviour may be potentiated. Further studies in our laboratory will investigate the effects of these and other growth factors on behaviour of other cells involved in the wound response such as keratinocytes.

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222P GLUTAMATE RECEPTORS AND SYNAPTIC PLASTICITY

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The discovery of multiple glutamate receptor subtypes and the development of selective antagonists for these receptors has enabled major insights into brain function in health and disease. We have been interested in the roles of glutamate receptors in synaptic transmission and plasticity in the hippocampus; in particular in the processes of long-term potentiation (LTP). This model for studying synaptic plasticity is providing insights into plastic changes that occur in the brain during learning and memory and various pathological states, including epilepsy, excitotoxicity and recovery from neuronal injury. Here, I shall summarise the current state of knowledge of LTP mechanisms in the hippocampus.

Most studies have focussed on the CA1 region of the rat or mouse hippocampus. Here, LTP is induced via the transient activation of the NMDA subtype of glutamate receptor and is expressed as a persistent increase in the efficiency of synaptic transmission mediated via both the AMPA and NMDA receptor subtypes. Activation of NMDA receptors results in a highly localised Ca^{2+} transient and this is believed to result in the activation of kinases, in particular CaMKII. With respect to LTP of AMPA receptor-mediated synaptic transmission, there is evidence that CaMKII can directly phosphorylate AMPA receptors (on the GluR1 subunit) and this leads to an increase in the amount of current that permeates AMPA receptors in unit time. In addition, activation of CaMKII may cause the rapid insertion of new AMPA receptors to increase the physical number of receptors at synapses.

In addition to NMDA receptors, activation of metabotropic glutamate (mGlu) receptors is also involved in the induction of LTP at CA1 synapses. However, their role is more complex and not fully understood. One role has been found for mGlu5 receptors that, via activation of CaMKII and PKC, induce a form of metaplasticity, which negates further requirement for the synaptic activation of mGlu receptors – a so-called "molecular switch".

The mossy fibre pathway innervating CA3 neurons is unusual in that NMDA receptors are not involved in the induction of LTP at these synapses. Instead, recent evidence suggests that kainate receptors may act as the trigger. In this pathway PKA seems to be the major signalling molecule involved. Again there is also evidence for a role for mGlu receptors.

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223P CELLULAR AND SUBCELLULAR DISTRIBUTION OF GLUTAMATE TRANSPORTERS: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL IMPLICATIONS

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Brain tissue has a remarkable ability to accumulate glutamate. This ability is due to glutamate transporter proteins present in the plasma membranes of both glial cells and neurons. Five sodium and potassium coupled glutamate transporters have been cloned so far (GLAST, GLT, EAAC, EAAT4, EAAT5). GLT is the most abundant of these five. It dominates quantitatively in all CNS regions except in those (cerebellum, retina) where GLAST is the major glutamate transporter. Both GLT and GLAST are only found in astroglia in the normal and mature nervous system. It should, however, be noted that GLT is neuronal in retina and at early stages during development. Both GLT and GLAST have been found in cultured neurons. EAAC is expressed by both neurons and glia. EAAT4 is almost exclusively found in cerebellar Purkinje cells, while EAAT5 is a retinal protein. It is surprising that none of the five transporters are found in glutamatergic nerve terminals. Nevertheless, glutamatergic nerve terminals do display sodium dependent glutamate uptake activity. This uptake has a similar sensitivity to inhibition by dihydrokainate as the uptake mediated by GLT, but the uptake is mediated by glutamate transporters which are not recognized by anti-GLT antibodies. This implies that glutamatergic nerve terminals express a novel glutamate transporter. It seems likely that this transporter may play an important role in limiting intersynaptic cross-talk and that this is the transporter that reverses during ischemia.

Because glutamate uptake is the only (significant) glutamate removal mechanism, it is a major determinant of the spatiotemporal concentration profile of glutamate in the extracellular space following glutamate release and thereby a major determinant of glutamate receptor activation.

Although our understanding of the glutamate uptake system is still incomplete, the emerging picture is that of a highly complex and sophisticated system for the control of synaptic, extrasynaptic as well as intersynaptic activation of glutamate receptors. The glutamate transporters thereby appear to have more refined roles than simple transmitter removal and probably participate in the regulation of synaptic and extrasynaptic transmission. So far, malfunctioning of the glutamate uptake system has not been proven to be the primary cause of any human disease, but studies on transgenic mice show that glutamate transporter deficiency leads to disease. Further, there is now a substantial support for the notion that glutamate play secondary (aggravating) roles in several widely different human diseases.

In most cases, however, we know too little about the roles of the glutamate uptake system. This lack of understanding represents an unknown factor in the modeling of pathological events in most major neurological diseases as well as in several non-neurological diseases.

224P METABOTROPIC GLUTAMATE RECEPTORS AS THERAPEUTIC TARGETS IN EPILEPSY.

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Acquired epilepsy in man (e.g. complex partial seizures associated with mesial temporal sclerosis) and in rats (e.g. amygdala kindled seizures) is associated with altered function of metabotropic glutamate receptors. Group I receptor responses are markedly potentiated in kindled rat amygdala. Group I receptor agonists, such as ACPD, (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid, and DHPG, 3,5-dihydroxyphenylglycine, injected focally into the thalamus or hippocampus induce seizure activity and focal neurodegeneration. Antagonists at mGlu1, such as LY367385, (+)-2methyl-4-carboxyphenylglycine, and AIDA, (R,S)-1-aminoindan-1,5-dicarboxylic acid, given intracerebroventricularly, (icv), block sound-induced seizures in DBA/2 mice and spike and wave discharges in lethargic mice (a model for absence attacks). The selective mGlu5 antagonists MPEP, 2-methyl-6-(phenylethynyl)-pyridine, and SIB1893, (E)-6-methyl-2-styryl-pyridine, most potently block seizures induced by CHPG, (R,S)-2-chloro-5-hydroxyphenylglycine, an mGlu5 selective agonist. They are, however, also systemically active against convulsions induced by DHPG, and against sound-induced convulsions in DBA/2 mice and spike and wave discharges in lethargic mice.

Various changes have been described in the function of Group II and III receptors in the amygdala and hippocampus of kindled rats; decreases in the function of Group III receptors have been described in the kindled rat hippocampus, and the human hippocampus with mesial temporal sclerosis. Group III agonists with little selectivity within the group, such as LAP4, L(+)-2-amino-4-phosphonobutyric acid, and LSOP, L-serine-O-phosphate given icv in mice or into the inferior colliculus in rats can have early excitatory effects and more prolonged anticonvulsant actions. A purer anticonvulsant effect is shown both by PPG, (R,S)-4-phosphonophenylglycine (with a high affinity for mGlu8) and by ACPT-1, (1S,3R,4S)-1-aminocyclopentane-1,2,4-tri-carboxylic acid, (a selective agonist for mGlu4α).

Group I antagonists and Group II and III agonists have significant anticonvulsant effects in rodent models of epilepsy. It remains to be determined which specific receptors are most importantly involved in the different syndromes of epilepsy, and whether any mGluR selective antagonist or agonist merits clinical trial as an antiepileptic or antiepileptogenic agent.

225P NMDA, AMPA AND KAINATE RECEPTOR ANTAGONISTS AS POTENTIAL ANTIEPILEPTIC DRUGS

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In 1982, Professor Brian Meldrum and his collaborators reported that a selective antagonist of NMDA receptors could protect against sound- and chemically-induced seizures in mice. This landmark discovery caused a paradigm shift in epilepsy research, moving the spotlight from GABA to glutamate, and igniting intense interest in the role of NMDA receptors in seizures. Some years later Meldrum was able to show that AMPA receptor antagonists also have anticonvulsant activity. Again the archetype changed! NMDA receptors had to share center stage and it was now clear that other types of glutamate receptors are also potential anticonvulsant targets, even those of the metabotropic type, as Meldrum has recently shown. Professor Meldrum's thoughts expressed eloquently in his research communications, public lectures and an occasional off the cuff suggestion have strongly guided our own work. We are grateful to have benefited from his leadership in this field, but I would be remiss to imply that our debt extends only as far as glutamate. Indeed, Meldrum along with his partner Dr. Astrid Chapman have touched virtually every area of anticonvulsant drug research.

In the first part of this talk, I will review the current status of efforts to develop NMDA and AMPA receptor antagonists for the clinical treatment of epilepsy. I will consider the potential of low-affinity uncompetitive NMDA antagonists and present recent data on the role of D-serine as an endogenous coagonist of NMDA receptors, which suggest that serine racemase inhibitors may some day find utility in epilepsy therapy and other conditions associated with excessive NMDA receptor activation.

I will then turn to the role of kainate receptors in epilepsy and epileptogenesis. The recent availability of decahydroisoquinolines that are selective antagonists of GluR5 kainate receptors has made it possible to demonstrate that these receptors participate in excitatory neurotransmission and in the regulation of GABA release. Our recent work has focused on the amygdala, which is among the most epileptogenic areas of the brain. A component of the excitatory synaptic response in the basolateral amygdala is mediated by GluR5 kainate receptors. *In situ* hybridization histochemistry has revealed that the amygdala contains a high density of GluR5 receptor subunit mRNA and by RT-PCR we have shown that this is composed of at least two splice forms. Using whole-cell voltage clamp recording in the amygdala slice, we characterized the GluR5 kainate receptor synaptic response and have shown that the synaptic current exhibits inward rectification (due to intracellular polyamines) and Ca^{2+} permeability.

In addition, I will discuss recent experiments implicating these receptors in a novel form of NMDA receptor independent synaptic plasticity, and *in vivo* studies that demonstrate a role for GluR5 kainate receptors in mediating limbic seizure activity.

Echoing Meldrum of nearly two decades ago, I conclude that glutamate receptors continue to be promising targets for antiepileptic drugs. Although the precise role of GluR5 kainate receptors is not yet well defined, these receptors exhibit characteristics suggesting that they may participate in seizures and epileptogenesis. While still in the green room, kainate receptors appear poised to move onto the stage in a third wave of research on ionotropic glutamate receptors and epilepsy.

226P PROSPECTS FOR AMPA/KAINATE ANTAGONISTS IN STROKE AND PAIN

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Glutamate receptors (NMDA, AMPA, kainate or metabotropic subtypes) are located on all neurones and mediate synaptic transmission throughout the mammalian brain and spinal cord. AMPA and kainate receptors are located both pre- and post-synaptically and are potential targets in several neurological conditions.

Until recently, selective kainate antagonists have not been available. The ability to screen compounds on cells recombinantly expressing various glutamate receptor subtypes together with innovative synthetic chemistry efforts, has allowed certain decahydroisoquinolines (eg. LY293558, LY294486, LY383884) with diverse antagonist profiles on AMPA (GLUA1-4) and kainate (GLUK5) subunits to be identified. We have used these compounds in animal models of stroke and pain to elucidate the role of AMPA and kainate receptors in these diseases.

In both global and focal ischaemia in gerbils and rats respectively, compounds with GLUK5 antagonism were more effective in reducing neurodegeneration than selective AMPA receptor antagonists. Such compounds also had a better therapeutic windows showing less sedation and ataxia at doses that were neuroprotective than selective AMPA antagonists.

Similarly in models of formalin-induced pain, compounds with GLUK5 antagonism proved to more efficacious and less sedating and ataxic than more selective GLUA1-4 antagonists. When examined in man, a mixed GLUA1-4 and GLUK5 proved to be analgesic against the hyperalgesia and allodynia that followed intradermal capsaicin injection. This beneficial effect occurred in man at doses that were without obvious ataxic or sedative side-effects.

Coupled with other data suggesting roles in other therapeutic indications, GLUK5 antagonists may have potential in neurological dysfunctions.

227P METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES INFLUENCE NEURONAL DEGENERATION THROUGH DIFFERENT MECHANISMS

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Metabotropic glutamate (mGlu) receptors form a family of eight subtypes (mGlu1 to –8), classified into three groups (mGlu1 and –5; mGlu2 and –3; and mGlu4, -6, -7 and –8) on the basis of their primary structure, pharmacological profile and transduction pathways.

All these subtypes (with the exception of mGlu6) have been implicated in the control of neuronal degeneration and survival, but the underlying mechanism is still unclear. Agonists of mGlu1 and –5 receptors (such as DHPG) can either facilitate or reduce neurodegeneration in culture depending on the paradigm of toxicity, the subunit composition of NMDA receptors, and the proportion between neurons and astrocytes. Interestingly, both effects can be observed when cultured cortical cells receive two consecutive applications of DHPG. While a single application of the drug amplifies NMDA toxicity, the second application consistently produces neuroprotection. This suggests the existence of an experience-dependent switch from facilitary into inhibitory group-I mGlu receptors in the control of excitotoxic death.

In contrast, mGlu1 and –5 receptor antagonists are consistently neuroprotective, but through different mechanisms. mGlu1 antagonists (such as CPCCOEt or LY367385) attenuate NMDA toxicity by enhancing GABAergic transmission, as shown by combining these drugs with GABA receptor antagonists or by measuring GABA release in freely moving animals. mGlu5 receptor antagonists (such as MPEP, SIB1757 or SIB1893) would instead prevent postsynaptic mGlu5 receptors from amplifying NMDA currents through a mechanism of receptor-receptor interaction. Neuroprotection by mGlu2/3 receptor agonists involves a novel form of glial-neuronal interaction mediated by an enhanced glial production of TGF-ß. In particular, activation of glial mGlu3 receptors induces the *de novo* synthesis of TGF-ß1 through the activation of the MAP-kinase pathway. The MEK inhibitor, PD98059, prevents the neuroprotective activity of mGlu2/3 receptor agonists against NMDA toxicity. Finally, the use of cultures prepared from knock-out mice suggests that activation of mGlu4 receptors mediate a large component of the neuroprotective action of group-III mGlu receptor agonists, such as L-AP4 and PPG.

Based on these mechanisms, subtype-selective mGlu receptor agonists or antagonists may have differential applications in the experimental treatment of neurodegenerative disorders.

228P METHODOLOGIES AND RESULTS OF STUDIES OF COGNITIVE CHANGES IN OLD AGE

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The main questions in Cognitive Gerontology are "When do Mental Abilities begin to change"? "How rapidly do changes continue?" "Do all mental abilities change together or do some change earlier than others"? and "What factors accelerate and retard cognitive changes"?" Are there simple biological or psychometric indices that can act as markers to predict future rates of change"?

To answer these questions we may either make cross-sectional comparisons between groups of different ages or longitudinal comparisons, tracking the same individuals over a period of many years. Longitudinal studies are more efficient because they also allow initial successive cross-sectional comparisons between groups and so reveal, and can control for cohort effects. However they have two serious and hitherto unacknowledged drawbacks: longitudinal studies suffer from selective drop-out of older and frailer participants so that populations become steadily more "elite"as the study continues. Also they involve repeated administration of the same, or very similar cognitive tests and this results in progressive improvement with practice, even when inter-test intervals range from 2 to 8 years. Practice effects are also counter-intuitive, with older and less able showing greater improvement than younger and more able participants.

We discuss recent statistical techniques for detecting and adjusting for both selective dropout and practice effects. When these techniques are applied the questions with which we began are answered in the following ways: Age related changes begin at a very early age, becoming detectable between the ages of 18 and 35 years and more marked in the range between 49 and 65 years, accelerating rapidly thereafter. However changes in average performance of ageing cohorts are much less interesting than increases in variance between their members. Because of very marked individual differences in trajectories of age-related change differences between the most and least able members of cohorts sharply increase as they age.

Evidently differences in trajectories of ageing are influenced by a variety of factors, among these genetics, general health and presence or absence of specific pathologies, socio-economic advantage education and life-style, gender and lifetime exposure to biological risk factors. We discuss the absolute and relative contributions of these factors to rates of cognitive change, and also new evidence that particular simple measurements of sensory function and balance can account for up to 85% of age-related variance in cognitive status between individuals.